Molecular diagnosis and immunotherapy

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Purpose of review
To describe the recent insights of how molecular diagnosis can be useful to improve indication and selection of suitable allergens for specific immunotherapy and to increase its safety.

Recent findings
As specific allergen immunotherapy is allergen-specific, the identification of the disease-eliciting allergen is a prerequisite for accurate prescription of anti-allergic treatment. In areas of complex sensitization to aeroallergens or in hymenoptera venom allergy, the use of molecular diagnosis has demonstrated that it may change indication and selection of allergens for immunotherapy in a large proportion of patients when compared with the use of skin prick testing and/or specific IgE determination with commercial extracts. These changes in the prescription of immunotherapy after using molecular diagnosis have been demonstrated to be cost-effective in some scenarios. Some patterns of sensitization to grass or olive pollen allergens may identify patients with higher risk of adverse reaction during immunotherapy.

Summary
Molecular diagnosis, together with other tools and patients' clinical history, can help clinicians better select the most appropriate patients and allergens for specific immunotherapy and, in some cases, predict the risk of adverse reactions.

Keywords
allergens, component resolved diagnosis, immunotherapy, molecular diagnosis

INTRODUCTION

Molecular diagnosis used in allergy seeks to define the allergen sensitization of a patient at the molecular level by measuring specific IgE to purified natural or recombinant allergens. Molecular diagnosis can improve diagnostic accuracy (specificity), resolve cross-reactivity from true co-sensitization, resolve low-risk markers from high-risk markers (biomarkers), and also improve indication and selection of suitable allergens for specific immunotherapy (SIT) [1]. As SIT is allergen-specific, the identification of the disease-eliciting allergen is a prerequisite for accurate prescription of treatment. This fact can be especially useful in the case of patients sensitized to multiple aeroallergens or hymenoptera venoms demonstrated by traditional methods using allergen extracts containing a mixture of allergenic and nonallergenic moieties by skin tests or by measuring specific IgE in serum. This approach will have a limited usefulness in patients if vaccines are prepared with all the allergens producing positive results in skin tests, as is common practice in the Americas [2,3]. However, the European Academy of Allergy and Clinical Immunology (EAACI) [4] recommends extracts for immunotherapy with few allergens. A recent review [5] has demonstrated a dose–response relationship for clinical efficacy of immunotherapy; therefore, any approaching seeking to reduce the number of allergens in the extract used for SIT may be very useful for increasing the efficacy of immunotherapy.

This review focuses only on the clinical utility of allergenic molecules that are currently available in commercial tests (Table 1) to improve indication and selection of suitable allergens for specific immunotherapy and to increase its safety.

METHODS TO MEASURE SPECIFIC IGE TO PURIFIED OR RECOMBINANT ALLERGENS

IgE to purified or recombinant allergens is usually measured on a fluorescence enzyme immunoassay. At present, three companies ImmunoCAP

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KEY POINTS

- Molecular diagnoses increase the accuracy of allergy diagnosis.
- Molecular diagnosis may help clinicians to select suitable allergens for specific immunotherapy.
- The use of molecular diagnosis may be cost-effective in a better selection of patients for immunotherapy.
- Some patterns of sensitization to allergens may predict the risk of adverse reaction to immunotherapy, and therefore increase its safety.

(Thermofisher-Scientific, Uppsala, Sweden), ImmuLite (Siemens AG, Erlander, Germany), and HyTech (Garden Grove, California, USA) offer the possiblitiy of measuring specific IgE to purified or recombinant allergens on singleplex platforms. The most extensive catalog of these allergens belongs to Thermofisher-Scientific. This company offers a unique system able to detect IgE to up to 112 components at the same time. This platform is a microarray-based assay [Immuno Solid phase Allergen Chip (ISAC)]. This is a miniaturized immunoassay platform in which allergen components are immobilized in a microarray. Only 30 μl of serum or plasma is needed, and both capillary and venous blood sampling can be used. Using a standard calibration curve, results are reported within a dynamic range of 0.3–100 ISU-E (ISAC standardized units) giving a semiquantitative indication of IgE antibody levels. Unlike these, singleplex systems are more quantitative. Due to differences in assay and measurement technology, these ISU-E units differ from kU/l given in ImmunoCAP results and therefore are not interchangeable, although a certain correlation has been

Table 1. Commercially available purified natural or recombinant allergens and their utility for specific immunotherapy

Sources	Allergens of genuine sensitization	Allergens with cross-reactivity
POLLEN		
Ragweed	Amb a 1	
Mugwort	Art v 1 Art v 3	Art v 3 (LTP)
Parietaria, wall pellitory	Par j 2	Par j 2 (LTP)
Russian thistle or saltwort	Sal k 1	
Goosefoot	Che a 1	
Timothy	Phl p 1 Phl p 5 Phl p 6	Phl p 4 (berberine) Phl p 7 (polcalcin) Phl p 11 (trypsin inhib.) Phl p 12 (profilin)
Bermuda grass	Cyn d 1	
Alder	Aln g 1	Aln g 1 (PR 10)
Birch	Bet v 1	Bet v 1 (PR10) Bet v 2 (profilin) Bet v 4 (polcalcin)
Olive	Ole e 1 Ole e 7 Ole e 9	
Japanese cedar	Cry j 1	
Cypress	Cup a 1	
Plane tree	Pla a 1 Pla a 2	Pla a 3 (LTP)
Latex	Hev b 1, Hev b 3, Hev b 5, Hev b 6.01, Hev b 6.02, Hev b 11	Heb v 8 (profilin) Hev b 11 (chitinase)
MITES		
House dust mite pyroglyphidae	Der p 1, Der p 2 Der f 1, Der f 2	Der p 10 (trpomyosin)
Blomia tropicalis	Blo t 5	
Lepidoglyphus destructor	Lep d 2	
PET DANDERS		
Cat	Fel d 1 Fel d 4	Fel d 3 (albumin)
Dog	Can f 1, Can f 2, Can f 5	Can f 3 (albumin)
Horse	Equ c 1	Equ c 3 (albumin)
HYMENOPTERA VENONS		
Bee	Api m 1, Api m 4	CCD (carbohydrate determinants)
Wasp	Ves p 1, Ves p 5 (common wasp) Pol d 5 (paper wasp)	CCD

observed. Several studies [6–10] have analyzed the reproducibility of this technique and have made a comparison with other methods of measuring specific IgE. Nevertheless, there is a general agreement that the reproducibility of ISAC is acceptable, but special attention is recommended for low specific IgE levels (0.3–1 ISU) in which increased variability has been observed. However, ISAC is not currently recommended for monitoring sensitization but can be useful to improve indication and selection of suitable allergens for specific immunotherapy and to increase its safety.

UTILITY OF MOLECULAR DIAGNOSIS IN SPECIFIC ALLERGENS

The most common allergens used in immunotherapy are explained in the following section.

Mites allergens

The use of the specific allergens Der p 1, Der p 2, Lep d 2, or Blo t 5 may help to distinguish true sensitization to pyroglyphidae mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*) from minor mites such as *Lepidoglyphus destructor* or *Blomia tropicalis* in some greographical areas [11]. For example, around 60% of patients in some Atlantic regions of the Iberian peninsula are sensitized to *Dermatophagoides spp* and *Lepidoglyphus d*, and almost 10% are monosensitized to the minor mite [12]. Thanks to the use of specific markers of sensitization to these mites, it was possible to accurately diagnose a true single sensitization to pyroglyphidae mites or *Lepidoglyphus d*. from a double sensitization, with clear consequences if SIT is decided. In tropical or sub-tropical areas, *Blomia tropicalis* is an important allergen and Blo t 5 can differentiate a true co sensitization from a cross-reactivity phenomenon, as major and minor mites have partial cross-reactivity [13,14]. This fact cannot be evidenced using skin test with whole mite extracts [15].

Tropomyosin has been considered to be responsible for cross-reactivity between crustaceans and other arthropods such as dust mites or cockroach and nematodes [16]. In fact, tropomyosins from dust mites and other arthropods have a shared sequence identity of about 75–80%. However, recent publications have shown than sensitization to tropomyosins is a good marker of clinical sensitivity to crustaceans but is not a marker of sensitization to mites [17–19]. Nevertheless, cross-reactivity between mites and shrimp does exist, but is due to other allergens than tropomyosins such as α-Actinin and Ubiquitin [20]. Therefore, and from a pragmatic point of view, in patients with clinical allergic reactions to crustaceans and with positive skin prick test (SPT) to mites, determination of markers of specific sensitization to mites is recommended.

Pet allergens

Many patients are poly-sensitized to several pet allergens using commercial extracts, but the clinical history is often inconclusive. This may be due in part to cross-reactivity phenomena between allergens contained in different extracts such as serum albumins of cat (Fel d 2), dog (Can f 3), cow, and horse (Equ c 3). All of these have a large wide cross-reactivity [21–23] and may be responsible for multiple positive reactions with conventional extracts. Thus, molecular diagnosis may clarify the relevant sensitization along with clinical history.

The detection of IgE to the major species-specific allergens Fel d 1, Fel d 4 from cat, Can f 1, Can f 2 and Can f 5 from dog, and Equ c 1 from horse together with specific albumins (Fel d 2, Can f 3, Equ c 3) will lead to proper diagnosis and correct SIT selection.

In conventional commercial extracts used for diagnosis, some allergens can be poorly represented because of the biological variability of the allergen source. For instance, Can f 5, a prostate-derived allergen produced by male dogs, is a dog allergen responsible for sensitivity in up to 38% of dog-allergic patients [24] (15% in the author's experience). This fact could explain why some patients sensitized to dog extracts may tolerate female dogs. Allergen extracts used in skin tests, however, typically use dog hair as an allergen source. As a result, these skin tests may routinely fail to identify patients monosensitized to Can f 5, likely due to its low concentration in dog hair. Determination of the IgE response to Can f 5 using molecular diagnosis diagnostics may enhance the accuracy of dog allergy diagnosis; if SIT is decided, the extract used should contain enough Can f 5.

Pollen allergens

Increasing the accuracy of diagnosis in pollen-allergic patients is a clinical challenge for specialists, mainly in areas where several pollens coexist. In such cases, it is important for the clinician to know whether a patient is co-sensitized to several allergen sources and needs SIT for each, or whether the patient is sensitized to several sources because of sensitization to cross-reactive components [e.g. profilins, polcalcins, lipid transfer proteins (LTPs), PR10] in each of the suspected allergen sources. For instance, a patient who is primarily sensitized to grasses may also test positive for birch, olive, or latex using SPT [25]. This cross-reactivity occurs

because all these extracts used in SPT contain profilin (rBet v 2, nOle e 2, Hev b 8), which are largely similar to those in grass (e.g. Phl p 12). Sensitization to Hev b 8 (profilin) seems to be clinically irrelevant and not related to clinical latex reactions; in this case, other relevant latex allergens should be tested (Hev b 1, Hev b 5, Hev b 6). Otherwise, sensitization to profilin has been associated with more severe respiratory symptoms in grass-allergic patients [26]. The clinical relevance of profilin as a respiratory allergen remains unknown, but a recent article showed than profilin may induce allergic respiratory symptoms in nasal and bronchial challenge tests, suggesting that profilins may be a potentially relevant respiratory allergen in which patients are exposed to high levels of grass pollen [27].

Molecular diagnosis using recombinants or purified allergens can partially solve this problem and improve the diagnosis of allergy. A recent publication [28▪▪] tackled how molecular diagnosis can change allergen-specific immunotherapy prescription in a complex pollen area. The aim of this study was to assess whether molecular diagnosis of pollen allergy leads to changes in the indication and allergen prescription of SIT when compared with skin prick testing with commercial extracts. It showed that in 54% of patients, there was a disagreement in indication or selection of allergens to be used in SIT before and after ISAC results. When analyzing concordance of SPT results with recombinants of purified allergens, the authors also found some degree of disagreement that varied from 40% with plane extract and positivity to Pla a 1 and/or Pla a 2 to 16% with grass extract and positive reaction to Phl p 1 and/or Phl p 5. This great discrepancy makes the case for the usefulness of molecular diagnosis, at least in areas of complex sensitization to pollen, as a means of facilitating accurate prescription of pollen immunotherapy. A post hoc pharmacoeconomic analysis using a simulation model of this study [29] showed that patients undergoing SIT using the strategy of skin tests + MD have a higher quality of life, and this can be quantified in 0.08 quality-adjusted life years (QALYs gained per patient per year). From a payer's perspective, in this population (141 patients), the total cost associated with testing every patient with molecular diagnosis using the ISAC platform was €31,020; however, if the CAP platform was used with 10 molecules, the cost was €14,100. The calculated costs associated with an incorrect SIT indication in 49 patients was €75,852. The net savings created by use of molecular diagnosis in this study was €44,020 (€318 per patient) or €61,752 (€438 per patient). In conclusion, the costs associated with testing with molecular diagnosis are justified by the substantial savings obtained by avoiding incorrect SITs and the QALYs gained.

Hymenoptera venom allergy

Diagnosis of hymenoptera venom allergy based on a clinical history and skin tests or specific IgE with whole venom extracts may result in some misdiagnosis due to cross-reactivity phenomena. Molecular diagnosis may help to choose a more accurate immunotherapy as major allergens rPol d 5, rVes v 5, Ves p 1, rApi m1 and the cross-reactive carbohydrate determinants may help to discriminate single from double sensitization to hymenoptera venoms [1,30▪▪,31].

Safety of immunotherapy

Concerning safety aspects of SIT and molecular diagnosis, an increasing risk of systemic reactions during immunotherapy with olive extract have been described in patients sensitized to Ole e 9 and Ole e 7 together with Ole e 1 [12]. Recently, we (manuscript in preparation) found that local or systemic reactions during the build-up phase of a trial (EudraCT#2011–000057–23) on the safety of subcutaneous SIT with a grass pollen extract (Avanz, ALK-Abello) were significantly associated with sensitization to a higher number of grass allergens and sensitization to Phl p 1 + Phl p 5 and/or Phl p 12. In this trial, 192 patients in Spain with rhinoconjunctivitis and/or asthma induced by grass pollen were included. Therefore, some patterns of sensitization to grass pollen could be considered risk markers for the development of adverse reactions during the build-up phase of immunotherapy in the studied population.

CONCLUSION

Defining the allergen sensitization of a patient at the molecular level by measuring specific IgE to purified natural or recombinant allergens (MD) can help clinicians to better select patients and allergens for prescribing specific immunotherapy. As demonstrated in some scenarios, this approach can be cost-effective. Some allergen sensitization patterns may also predict the risk of adverse reactions to SIT.

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Conflicts of interest

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